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Synthesis of New Organophosphorus Pyrazole Derivatives as Human Myeloblastic Leukemia HL60 Cytotoxic Agents

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Abstract

Hexaphenylcarbodiphosphorane reacted with oxobutenyl-, and oxophenylpropenyl-pyrazolones to give oxaphosphinin, triphenylphosphaneylidenecyclobutenyl, (triphenylphosphaneylidene)-butadienyl and triphenyldihydrophosphetyl derivatives. Triphenylphosphane and triphenylphosphane oxide were also isolated and identified. Moreover, Japanese reagent reacted with the same pyrazolones to afford sulfido-oxathiaphosphinin products. Possible reaction mechanisms were considered and structured elucidations for the new products were based upon compatible microanalyticals and spectroscopic measurements. Fournewly synthesized compounds showed promising cytotoxicity against human myeloblasticleukemia HL60 cells with selectivity towards tumor cells when compared to non-tumor human fibroblasts BJ cells.

Keywords: phosphallene; bis-ylide; oxaphosphinin; Japanese reagent**;** myeloblasticleukemia HL60 cells

Introduction

Cancer is a global term related to a large category of diseases that may destroy all parts of the human body. In 2020, around 10 million are dramatic deaths worldwide [1]. One of the main causes of cancer death is lung (1.80 million deaths in 2020). On the other side, leukemia is the tenth of all cancer cases and accounts 3.5% of all cancer types that affect men more than women. Many methods of treating cancer are well developed according to the type of cancer disease, immunotherapy, radiation therapy, hyperthermia, surgery and chemotherapy. Chemotherapy is one of the important tools for cancer treatment [2]. There are many compounds that can be used and reported as anticancer drugs, and others are under investigation [3]till clinical trials [4].

In recent years, pyrazolones have a marked attention in many fields. 5-Pyrazolone skeletons are present in many natural products that have effective applications as anti-inflammatory [5, 6], analgesic [7], antioxidant [8], anticancer [9], anti-diabetic [10], and antimicrobial activities [11]. Some from the best FDA-based drugs is Morazone which acts as anti-inflammatory drug and Edaravone which is commercially available and treats neurological [12] and non-neurological diseases [13]. Moreover, Telin which is approved as anticancer drug [14]. Tepoxalin also contains pyrazole moiety and is used as anti-inflammatory, analgesic and is used in veterinary medicine to control pain [15], (Figure 1).

On the other hand, organophosphorus compounds are a big group of effective drugs [16]. Heterocyclic compounds containing organophosphorus moieties have an important role in organic chemistry, environmental chemistry, natural products, medicinal chemistry and pharmacological purposes [17-19]. Some of these compounds are alkylating agents and make DNA cross linking [20, 21]. Oxazaphosphorines are a class of organophosphorus anticancer drugs [22]. The most common drug in this group is cyclophosphamide which is used in the treatment of bone sarcoma and tumor of soft tissues [23]. Ifosfamide is used for the treatment of lung cancer, ovarian cancer and breast cancer whereas

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Trofosfamide targets the malignant tumor [24]. Moreover, Cidofovir is antiviral drug which interacts directly with viral DNA polymerase [25].

On the other side, oxaphosphinin derivatives have ubiquity in many biological systems that make a richness in the phosphorus chemistry [26]. Oxaphosphinin heterocyclic compounds containing sugar moiety are active ingredients against cerebral tumors [27]. Moreover, they are natural product analogues [28] and their coumarin analogues are bioactive against two types of leukemia tumors HL-60 and NALM-6 [29, 30].

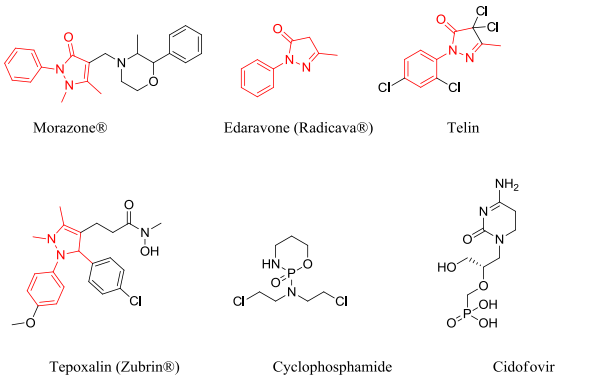


Figure 1: The most common pyrazolone and organophosphorus drugs

Experimental

1-Chemistry

All chemicals were supplied by either Fluka or Aldrich chemical companies and were used without further purification. Starting material was prepared according to literature survey [31]. All melting points are uncorrected and were taken in open capillary tubes using Electro thermal digital melting point apparatus 9100 (Electro-Thermal Engineering Ltd., Essex, United Kingdom). Elemental microanalyses were carried out at Microanalytical Unit, Central Services Laboratory, National Research Centre, Dokki, Giza, Egypt, using Vario Elementar and were found within ±0.4% of the theoretical values. **FT-IR** spectra were recorded with a Perkin-Elmer Frontier. Routine NMR spectra were recorded at room temperature on a Bruker Avance TM 300 MHz and Joel TM 500 MHz spectrometers as solutions in dimethyl sulfoxide (DMSO-d6). The mass spectra were measured with a GC Finnigan MAT SSQ-7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds were made by Thin Layer Chromatography (TLC) on silica gel-precoated aluminum sheets (Type 60, F 254, Merck, Darmstadt, Germany) with eluent of petroleum ether (b.r. 60-80 °C)/ethyl acetate or acetone and the spots were detected by exposure to UV lamp at λ_{254}nanometer for a few seconds. The chemical names given for the prepared compounds are according to the IUPAC system. The reported yields are based upon pure materials isolated by column chromatography with eluent of petroleum ether (b.r. 60-80°C)/ethyl acetate or acetone. Solvents were dried/purified according to conventional procedures.

Reaction of hexaphenylcarbodiphosphor-ane (2) with1,5-dimethyl-4-(3-oxobut-1-en-1-yl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (1a) and/ with 1,5-dimethyl-4-(3-oxo-3-phenylprop-1-en-1-yl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (1b).

To a solution of **(2)** [32] (1.072 g, 0.002mol) in 20 mL dry toluene, was added a solution of **(1a)** and/ or **(1b)** in 30 mL toluene. The reaction mixture was refluxed for 3 h in case of **1a** and 20 h in case of **1b** (TLC control). The solvent was distilled off under reduced pressure and the residue was subjected to column chromatography using pet.ether 60-80°C/ethylacetateas an eluent to give **3b**, **4a,b, 5a,b, 7a,b** and **9**alongwith triphenylphosphane (m.p. and mix. m.p. 79°C) and triphenylphosphane oxide (m.p. and mix. m.p. 151°C).

1,5-Dimethyl-2-phenyl-4-(2,2,2,6-tetra- phenyl-3-(triphenyl-λ^5-phosphaneylidene)-3,4-dihydro-2H-1,2 λ^5-oxaphosphinin-4-yl)-1,2-dihydro-3H-pyrazol-3-one **(3b).**

This product was recrystallized from cyclohexane as colourless crystals, yield 20%, m.p:122-124°C, IR (KBr, *cm*$^{-1}$): $\tilde{\nu}$ = 1720 (C=O),1594 (C=P).^{1}H NMR (300 MHz, d_6- DMSO, δ, ppm): 2.82 (s, 3 H, CH_3), 3.13 (s, 3 H, *N*-CH_3), 4.17 (dd, 1 H, CH, J_{HH}, J_{PH} = 9 Hz), 7.20 (d, 1H, CH), 7.25-8.35 (m, 40 H, H-arom.).^{13}C NMR- (75 MHz, d_6-DMSO, δ, ppm): 155.98 (C=O), 132.94-126.15 (arom.-C), 39.5 (CH_3).^{31}P NMR (100 MHz, d_6-DMSO): δ 10.124, 30.27 ppm. MS m/z (%): 840 [[M^{-1}-CH_3]$^+$, 2], 826 [[M-CO]$^+$, 10], 593 [[M-Ph_3P]$^+$, 24]. Anal. Calcd.for: $C_{57}H_{48}N_2O_2P_2$ (854.97). Calcd: C, 80.08; H, 5.66; N, 3.28; P, 7.25. Found: C, 79.97; H, 5.58; N, 3.21; P, 7.18.

1,5-Dimethyl-4-(6-methyl-2,2,2-triphenyl-2H-1,2λ^5-oxaphosphinin-4-yl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one **(4a)**

This product was recrystallized from cyclohexane as colourless crystals, yield 20%, m.p: 165-167°C.^{1}H NMR (300 MHz, d_6- DMSO, δ, ppm): 2.12 (s, 3 H, CH_3), 2.14 (s, 3 H, CH_3), 3.04 (s, 3 H, *N*-CH_3), 3.08 (s, 1 H, CH), 5.77 (d, 1H, CH, J_{PH} = 15 Hz), 7.25-7.48 (m, 20 H , H-arom.). ^{13}C NMR (75 MHz, d_6-DMSO, δ, ppm): 163.25 (C=O), 155 (C=P), 113.89-130.37 (arom.-C), 36.37 (*N*-CH_3), 31.66 (*N*-C-CH_3), 23.28 (O-C-CH_3). MS m/z (%): 529 $[M-H]^+$. Anal. Calcd. For $C_{34}H_{31}N_2O_2P$ (530.61). Calcd: C, 76.96; H, 5.89; N, 5.28; P, 5.84 Found: C, 76.87; H, 5.85; N, 5.19; P, 5.77.

1,5-Dimethyl-2-phenyl-4-(2,2,2,6-tetraphen- yl-2H-1,2λ^5-oxaphosphinin-4-yl)-1,2-dihyd- ro-3H-pyrazol-3-one **(4b)**

This product was recrystallized from cyclohexane as colourless crystals, yield 20%, m.p: 144-146 °C.^{1}H NMR (300 MHz, d_6-DMSO, δ, ppm): 2.60 (s, 3 H, CH_3), 3.007 (s, 3 H, *N*-CH_3), 6.91 (d, 1 H, CH, J_{PH} = 6 Hz), 7.26- 7.81 (m, 26 H, H-arom.).MS m/z (%): 590 $[[M-2H]^+$, 2]. Anal. Calcd. for: $C_{39}H_{33}N_2O_2P$ (592.68). Calcd: C, 79.04; H, 5.61; N, 4.73; P, 5.23. Found: 78.90; H, 5.59; N, 4.68; P, 5.12.

1,5-Dimethyl-4-(3-methyl-4-(triphenyl-λ^5-phosphaneylidene)cyclobut-2-en-1-yl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one **(5a)**

This product was recrystallized from cyclohexane as colourless crystals, yield 20%, m.p: 183-185°C, IR (KBr, cm^{-1}): $\widetilde{\nu}$ = 1653 (C=O),1490 (C=P).^{1}H NMR (300 MHz, d_6-DMSO, δ, ppm): 1.99 (s, 3 H, O-C-CH_3), 2.08 (s, 3 H, *N*-C-CH_3), 2.70 (dd, 1H, CH, J_{HH} ,J_{PH} = 10 Hz) 2.96 (d, 1 H, CHJ_{HH} = 10 Hz), 3.16 (s, 3 H, *N*-CH_3), 7.29-7.50 (m, 20 H , H-arom.). ^{13}C NMR (75 MHz, d_6-DMSO, δ, ppm): 164.75 (C=O), 160.27 (C=P), 122.87-135.40 (arom.-C), 36.09 (*N*-CH_3), 29.49 (N-C-CH_3), 23.39 (O-C-CH_3). MS m/z (%): 514 $[[M]^+$, 4]. Anal. Calcd. for $C_{34}H_{31}N_2OP$ (514.61). Calcd: C, 79.36; H, 6.07; N, 5.44; P, 6.02. Found: C, 79.29; H, 6.02; N, 5.39; P, 5.95.

1,5-Dimethyl-2-phenyl-4-(3-phenyl-4-(triph- enyl-λ^5-phosphaneylidene)cyclobut-2-en-1-yl)-1,2-dihydro-3H-pyrazol-3-one **(5b)**

Colourless crystals, yield 20%, m.p: 114-116°C, IR (KBr, cm^{-1}): $\widetilde{\nu}$ = 1653 (C=O), 1590 (C=P).^{1}H NMR (300 MHz, d_6- DMSO, δ, ppm): 2.49 (s, 3 H, *N*-C-CH_3), 2.60 (s, 3 H, N-CH_3), 2.98 (dd, 1 H, CH J_{HH}, J_{PH} = 12 Hz), 6.91 (d, 1H, CH J_{PH} = 12 Hz), 7.27-7.81 (m, 25 H, H-arom.). ^{13}C NMR (75 MHz, d_6-DMSO, δ, ppm): 164.09 (C=O), 138.08-122.97 (arom.-C), 35.22 (*N*-CH_3), 31.49 (CH_3). MS m/z (%): 577 $[M+H]^+$.Anal.Calcd.for: $C_{39}H_{33}N_2OP$ (576.68). Calcd: C, 81.23; H, 5.77; N, 4.86; P, 5.37. Found: C, 81.8; H, 5.72; N, 4.81; P, 5.27.

1,5-Dimethyl-4-(3-methyl-4-(triphenyl-λ^5-phosphaneylidene)buta-1,3-dien-1-yl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one **(7a)**

This product was recrystallized from cyclohexane as colourless crystals, yield 20%, m.p: 110-112°C, IR (KBr, cm^{-1}): $\widetilde{\nu}$ = 1655 (C=O),1485 (C=P).^{1}H NMR (300 MHz, d_6-DMSO, δ, ppm): 2.00(s, 3 H, O-C-CH_3), 2.04 (s, 3 H, *N*-C-CH_3), 3.35 (s, 3 H, N-CH_3), 7.43-7.77 (m, 22 H, H-arom.). MS m/z (%): 514 $[[M]^+$, 2]. Anal. Calcd. for $C_{34}H_{31}N_2OP$ (514.61). Calcd: C, 79.36; H, 6.07; N, 5.44; P, 6.02. Found: C, 79.29; H, 6.02; N, 5.39; P, 5.95.

1,5-Dimethyl-2-phenyl-4-(3-phenyl-4-(triph- enyl-λ^5-phosphaneylidene)buta-1,3-dien-1-yl)-1,2-dihydro-3H-pyrazol-3-one **(7b)**

This product was recrystallized from cyclohexane as colourless crystals, yield 20%, m.p: 189-191°C, IR (KBr, cm^{-1}): $\widetilde{\nu}$ = 1653 (C=O),1500 (C=P).^{1}H NMR (300 MHz, d_6- DMSO, δ, ppm): 2.49 (s, 3 H, CH_3), 3.12 (s, 3 H, *N*-CH_3), 6.93-7.98 (m, 27 H , H-arom.) ^{13}C NMR (75 MHz, d_6- DMSO, δ , ppm): 168.03 (C=O), 122.30-132.08 (arom.-C), 77.08 (C=C=P), 29.70 (CH_3), 21.44 (CH_3).MS m/z (%): 575 $[[M-H]^+$, 2]. Anal. Calcd. for: $C_{39}H_{33}N_2OP$ (576.68). Calcd: C, 81.23; H, 5.77; N, 4.86; P, 5.37. Found: C, 81.8; H, 5.72; N, 4.81; P, 5.27.

4-(2-Benzoyl-1,1,1-triphenyl-1,2-dihydro-1λ^5-phosphet-3-yl)-1,5-dimethyl-2- phenyl-1,2-dihydro-3H-pyrazol-3-one **(9)**

This product was recrystallized from ethyl acetate as colourless crystals, yield 20%, m.p: 283-285°C, IR (KBr, cm^{-1}): $\widetilde{\nu}$ =1709 (C=O, pyrazolone), 1652 (Ph-C=O).^{1}H NMR (300 MHz, d_6-DMSO, δ, ppm): 3.19 (s, 3 H, CH_3), 3.32 (s, 3 H, *N*-CH_3), 3.38 (d, 1 H, CH, J_{PH} = 6 Hz), 7.51-7.58 (m, 26 H, H-arom.).^{13}C NMR (75 MHz, d_6-DMSO, δ, ppm): 205.01 (Ph-C=O), 152.97(pyrazolon), 124.03-142.07(arom.-C), 37.34 (*N*-CH_3), 20.96 (CH_3).MS m/z (%): 591 $[[M-H]^+$, 2].Anal.Calcd.for: $C_{39}H_{33}N_2O_2P$ (592.68). Calcd: C,

79.04; H, 5.61; N, 4.73; P, 5.23. Found: 78.90; H, 5.59; N, 4.68; P, 5.12.

The reaction of Japanese reagent (10) with 1,5-dimethyl-4-(3-oxobut-1-en-1-yl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (1a) and/ with1,5-dimethyl-4-(3-oxo-3-phenyl- prop-1-en-1-yl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (1b).

A mixture of compound **1a** (0.256 g, 0.001 mol) or compound **1b** (0.318 g, 0.001mol) and *Japanese reagent* **10** (0.408 g, 0.001 mol) in (30 mL) dry toluene was refluxed for 5 h in case of **1a** and 8 h in case of **1b** (TLC control). The precipitates of compounds **11a** and **11b** were formed, respectively.

1,5-Dimethyl-4-(6-methyl-2-(phenylthio)-2-sulfido-4H-1,3,2-oxathiaphosphinin-4-yl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one **(11a)**.

This product was recrystallized from ethyl acetate as brown crystals, yield 75%, m.p: 310-312°C, IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1720 (C=O).^{1}H NMR (300 MHz, d_6-DMSO, δ, ppm): 1.85(s, 3 H, O-C-CH_3),2.19 (s, 3 H, *N*-C-CH_3), 3.20 (s, 3 H, *N*-CH_3), 3.61 (d, 1 H, CH,J_{HH} = 6 Hz),4.13 (d, 1 H, CH, J_{HH} = 6 Hz), 7.15-7.70 (m, 10 H, H-arom.). ^{31}P NMR (100 MHz, d_6- DMSO): δ 21.03 ppm. MS m/z (%): 460 [[M]$^+$, 7]. Anal. Calcd. for: $C_{21}H_{21}N_2O_2PS_3$ (460.56). Calcd: C, 54.77; H, 4.60; N, 6.08; P, 6.73; S, 20.88. Found: 54.69; H, 4.59; N, 6.01; P, 6.68.

1,5-Dimethyl-2-phenyl-4-(6-phenyl-2-(phen-ylthio)-2-sulfido-4H-1,3,2-oxathiaphosphin- in-4-yl)-1,2-dihydro-3H-pyrazol-3-one **(11b)**.

This product was recrystallized from ethyl acetate as dark brown crystals, yield 70%, m.p: 136-138°C. ^{1}H NMR (300 MHz, d_6- DMSO, δ, ppm):2.28 (s, 3 H, CH_3), 3.11 (s, 3 H, *N*-CH_3), 3.68 (d, 1 H, CH,J_{HH} = 6 Hz), 7.15-7.83 (m, 16 H, H-arom.).^{31}P NMR (100 MHz, d_6-DMSO): δ.21.79 ppm. MS m/z (%): 522 [[M]$^+$, 2].Anal .Calcd. for: $C_{26}H_{23}N_2O_2PS_3$ (522.64). Calcd: C, 59.75; H, 4.44; N, 5.36; P, 5.93; S, 18.40. Found: C, 59.67; H, 4.41; N, 5.29; P, 5.89; S, 18.37.

2- **Determination of cell viability**

Human myeloblastic leukemia HL60, human lung carcinoma A549 and human non-tumor skin fibroblasts BJ cells were obtained from American Type culture collection (Manassas, VA). A549 and BJ cells were maintained in DMEM-F12 medium, whereas HL60 was maintained in RPMI 1640 medium. All media were supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin and 100 µg/mL streptomycin(Invitrogen, CA).

Cell viability was determined using MTT assay as described previously [33]. Briefly, cells (25-35 x 10^3per well) were incubated with increasing concentrations of each compound in serum free media for 48 h in a humidified carbon dioxide incubator at 37°C. Thereafter, MTT solution (0.5 mg/mL) was added to each well for 4 h. Isopropanol was used to dissolve the formed blue crystals in A549 and BJ cells, whereas 10% SDS in 0.04 N HCL solution was used for HL60 cells. Finally, the formed colour was measured at 570/690 nm using micro-plate reader (Bio-Tec, Instruments, Winooski,VT). We used doxorubicin HCL (Adricin®, 100µg/mL, EIMC United Pharma, Egypt) as a positive control that showed more than 85% cytotoxicity for all used cell lines.

Statistical analysis was carried out using One way ANOVA followed by Student-Newman-Keuls post hoc test using Sigma Stat 3.5 program for Windows, Systat software Inc. (San Jose, CA). Median inhibitory concentration (IC_{50}) was determined using a semi log figure, the best fitting line was drawn, and the concentration that causes 50% loss of viability was determined. Whereas the selectivity index (SI) was calculated by dividing the $IC_{50}$for non-tumor BJ cells over the $IC_{50}$for HL60 cells.

Results and Discussion

1-Chemistry

Herein, synthesis of new biologically active heterocyclic [34-39] and carbocyclic containing phosphorus and sulphur compounds and their biological screening as anticancer agents have been investigated. Hexaphenylcarbodiphosphorane **(2)** is an interesting organophosphorus reagent; due to it has two resonance structures $2A \rightleftharpoons 2B$.

Reaction of hexaphenylcarbodiphosphorane **(2)** with1,5-dimethyl-4-(3-oxobut-1-en-1-yl)-2-phenyl-1,2-dihydro-*3H*-pyrazol-3-one **(1a)** proceeded in boiling dry toluene to give new phosphorelated compounds **4a**, **5a**, and **7a** that were assigned based on correct microanalytical and spectroscopic evidences. Triphenylphosphane and triphenylphosphane oxide were also isolated and identified. Compound, 1,5-dimethyl-4-(6-methyl-2,2,2-triphenyl-2H-1,2λ^5-

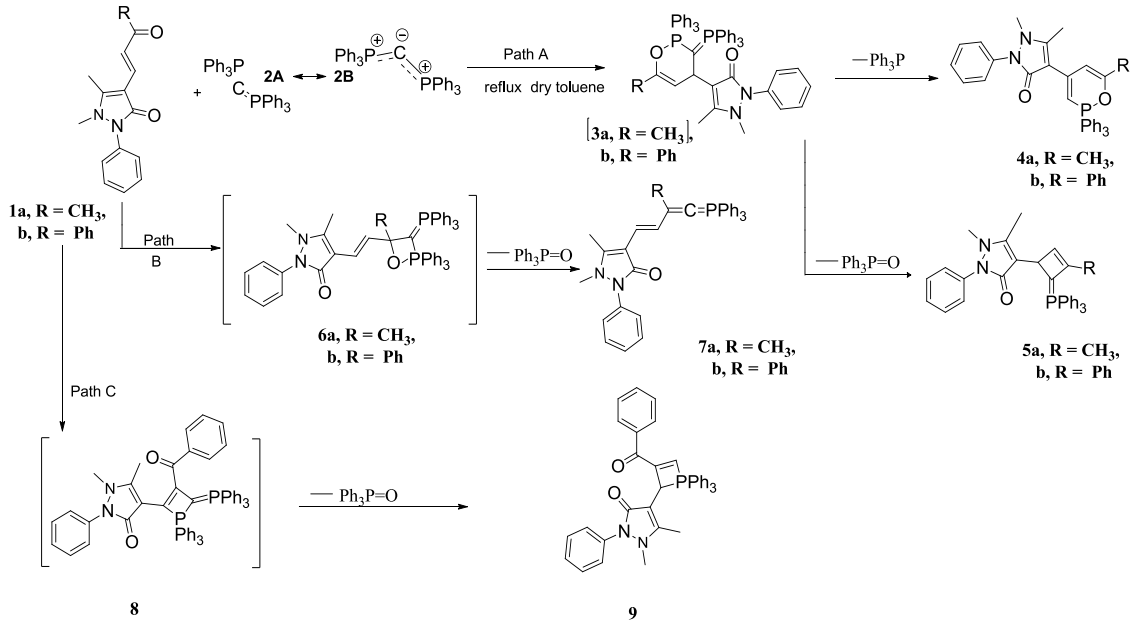
oxapho- sphinin-4-yl)-2-phenyl-1,2-dihydro-*3H*-pyrazol-3-one **(4a)** showed an ion peak at *m/z* 529 [M-H] in its mass spectrum. The ^{1}H-NMR spectrum of compound 1,5-dimethyl-4-(3-methyl-4-(triphenyl-λ^5-phos -phaneylidene)cyclobut-2-en-1-yl)-2-phenyl-1,2-dihydro-*3H*-pyrazol-3-one **(5a)** in $CDCl_3$ showed signals at δ1.99 (s, 3 H, O-C-CH_3), 2.08 (s, 3 H, *N*-C-CH_3), 2.70 (dd, 1H, CH, $^{3}J_{HP}$ = 10 Hz) 2.96 (d, 1 H, CHJ_{HH} = 10 Hz), 3.16 (s, 3 H, *N*-CH_3), 7.29-7.50 (m, 20 H, H-aromatics). The mass spectrum of 1,5-dimethyl-4-(3-methyl-4-(triphenyl-λ^5-phosphaneylidene)buta-1,3-dien-1-yl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one **(7a)** showed the molecular ion peak at *m/z* 514 $[M]^+$ corresponding to the molecular formula: $C_{34}H_{31}N_2OP$. Apparently, [4+2]-cycloaddition of compound **1a** to the ylidenephosphorane **2** would give intermediate **3a**. This is followed by *Hoffmann degradation via* removal of the good leaving group to produce **4a** [40]. Intermediate **3a** can also extrude triphenylphosphane oxide molecule to give **5a** (Path A).

[2+2]-Cycloaddition of the carbonyl function in **1a** to the C-P group in **2** would produce the unstable oxaphosphetane **6a** which can extrude triphenylphosphane oxide molecule to give 1,5-dimethyl-4-(3-methyl-4-(triphenyl-λ^5-phosphaneylidene)buta-1,3-dien-1-yl)-2-phenyl-1,2-dihydro-*3H*-pyrazol-3-one **(7a)** [41].

In the same manner, the 1,5-dimethyl-4-(3-oxo-3-phenylprop-1-en-1-yl)-2-phenyl-1,2-dihydro-*3H*-pyrazol-3-one **(1b)** was allowed to react with phosphallene**2** to give five products for which structures **3b, 4b, 5b, 7b** and **9** that were assigned upon correct analytical and spectroscopic measurements (see experimental part). Triphenylphosphane and triphenylphosphane oxide were also isolated and identified. The most evidence of **3b** is the presence of two signals atδ 10.124, 30.27 ppm in the ^{31}P NMR of compound **3b**.

Meanwhile, compound **3b** undergoes *Hoffmann degradation* to give 1,5-dimethyl-2-phenyl-4-(2,2,2,6-tetraphenyl-*2H*-1,2λ^5-oxaphosphinin-4-yl)-1,2-dihydro-*3H*-pyrazol-3-one **(4b)** along with triphenylphosphane. Moreover, the phosphopyran **3b** lost a molecule of triphenylphosphane oxide to give **5b**.

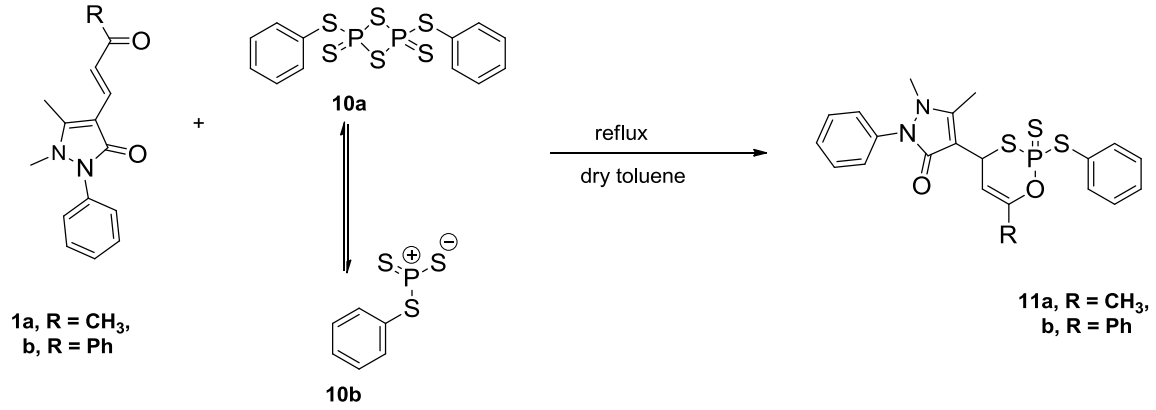
The path B can be expressed by [2+2]-cycloaddition of C=O of compound **1b** to C-P of the bisylide**2** to give the unstable intermediate oxaphosphetane **6b** [42] which after losing a molecule of triphenylphosphane oxide to give **7b**. Moreover, 4-(2-benzoyl-1,1,1-triphenyl-1,2-dihydro-1λ^5-phosphet-3-yl)-1,5-dimethyl-2-phenyl-1,2-dihydro-*3H*-pyrazol-3-one **(9)** can be obtained through [2+2]-cycloaddition of C=C bond of compound **1b** to C-P of diphosphorane **2** which acts as a base-catalyst [43-45]. This activates the chalcone double bond to form unstable intermediate **8** then expulsion of triphenylphosphane from the intermediate **8**. The most important features are the carbonyl group absorptions at δ 205.01 and 152.97 ppm in the ^{13}C NMR spectrum of compound **9 (**scheme 1).



Scheme (1)

On the other side, (2,4-bisthiophenyl-1,3,2,4-dithiaphosphetane-2,4-disulfide) (*Japanese reagent*, **10a**⇌**10b**) reacted with pyrazol-3-ones **1a** and **1b** in boiling toluene to give the new 1,5-dimethyl-4-(6-methyl-2-(phenylthio)-2-sulfido-*4H*-1,3,2-oxathia phosphinin-4-yl)-2-phenyl-1,2-dihy- dro-*3H*-pyrazol-3-one **(11a)** and 1,5-dimeth- yl-2-phenyl-4-(6-phenyl-2-(phenylthio)-2-sulfido-*4H*-1,3,2-oxathiaphosphinin -4-yl)-1,2-dihydro-*3H*-pyrazol-3-one **(11b)**, respectively.

Structural assignments for **11a** and **11b** were based upon correct microanalytical and spectroscopic evidences. Their ^{31}P NMR spectra (in DMSO) disclosed the presence of a singlet signal at δ 21.03 (for **11a**) and at δ 21.79 ppm (for **11b**). Apparently, the dipolar structure **10b** of the *Japanese reagent* can undergo [4+2]-cycloaddition with **1a**,**b** to afford six-membered ring oxathiaphosphinin derivatives **11a,b** (Scheme 2).



Scheme (2)

2-Effect on human tumor cell lines viability

In order to examine the possible biological effects of the newly synthesized compounds, we tested their effect on 2 human tumor cell lines viability, namely human lung carcinoma A549, and human leukemia HL60 cell lines. The compounds were dissolved in DMSO and increasing concentrations were incubated with each cell line for 48 h before the addition of MTT salt. As indicated in figures 2 and 3, the effect of the compounds was much more pronounced with human leukemia HL60 than human lung carcinoma A549 cells. However, only compound **7b** showed a high cytotoxic effect with A549 and the effect was significantly different when compared to the non-tumor BJ cells (Fig.3C).

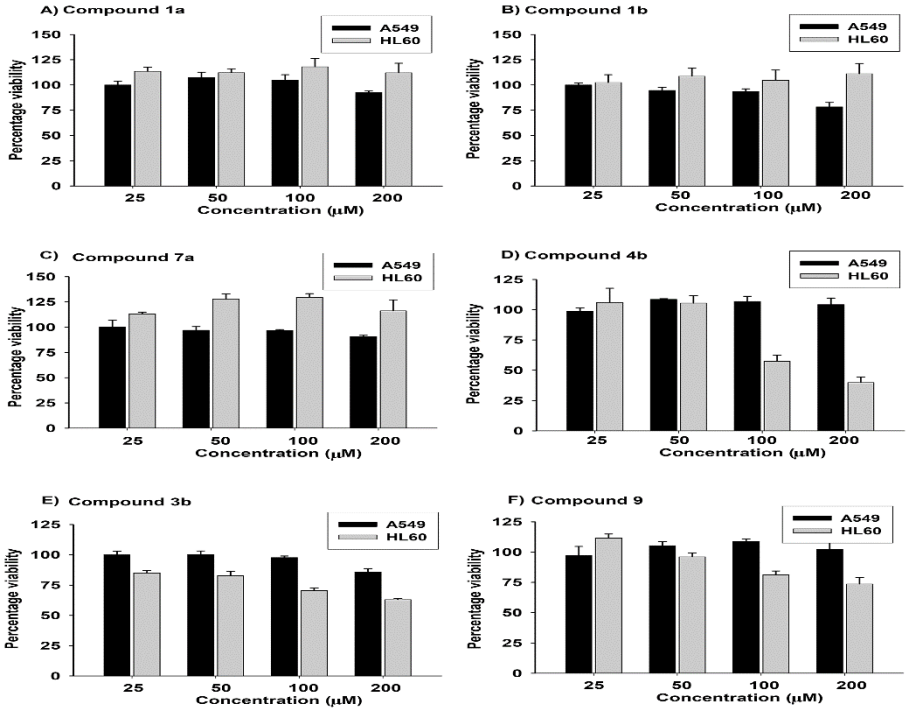


Figure 2: Effect of newly synthesized compounds on human lung A549, and human leukemia HL60 cell viability using MTT assay. Vertical bars represent percent of DMSO-treated control which was set as 100%± S.E.M. (n=3), n=3.

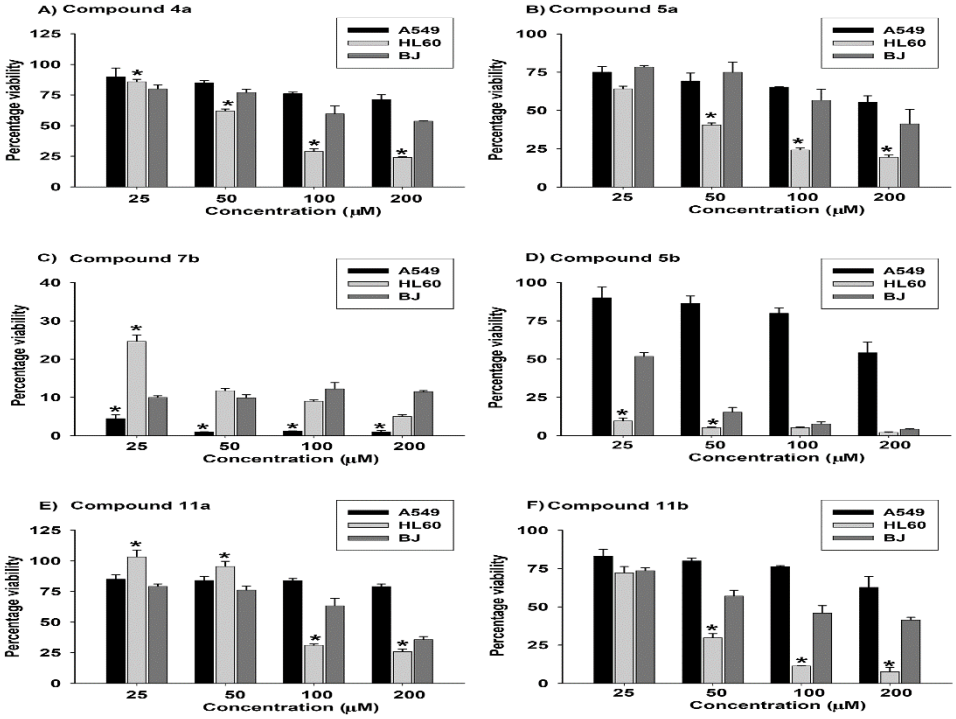


Figure 3: Effect of newly synthesized compounds on human lung carcinoma A549, and human leukemia HL60 cell viability. The effect of compounds was compared to their effect on non-tumor human skin fibroblasts BJ cell viability. Data were expressed as percent of DMSO-treated control which was set as 100% ± S.E.M. (n=3), (*) $P< 0.05$, significantly different from the same treatment on BJ cells.

Table 1: The median inhibitory concentration (IC_{50}) and selectivity index (SI) of most active compounds

Compound	IC_{50} (µM) toHL60 cells	IC_{50} (µM) toBJ cells	SI to BJ cells
5a	38.9	152.31	3.92
4a	71.12	215.1	3.02
11b	37.09	98.93	2.67
11a	97.09	147.26	1.52
5b	< 25	17.44	NA
7b	< 25	< 25	NA

To determine the most active compound(s) on HL60, median inhibitory concentration (IC_{50}) was estimated for each compound for HL60 and BJ cells with calculation of the selectivity index (SI= IC_{50} to BJ cells / IC_{50} to HL60 cells) (Table 1).

Structure-activity relationship

Regarding to the most active compounds are **4a**, and **5a** with SI of 3.02 and 3.9, respectively. The six-membered ring derivative **4a** is potent against HL60 cells and BJ cells due to the presence of oxaphosphinin group and two methyl groups in the molecular structure of compound **4a** which plays an important role in the activity due to it increases the potency of the drug and stops the metabolism meanwhile it increases the duration of action of certain drug. Moreover, the presence of *N*-methyl group that significantly increasing the activity four times than the compound which lacks this group [46]. In addition, compound **5a** is also efficient on these cell lines due to the formation of phosphranylidene cyclobutane group in the molecular structure of **5a** [47] that may responsible for the activity of this compound. On the other hand, compounds **11b** and **11a** come next with SI of 2.7 and 1.5, respectively due to the presence of oxathiaphosphinin groups in the molecular structure of these compounds make increasing their activity (Table 1).

The most active compounds are **4a**, and **5a** with SI of 3 and 3.9, respectively. Compounds **11b** and **11a** come next with SI of 2.7 and 1.5, respectively (Table 1). SI can be used to determine the selectivity of the compound to tumor cells than non-tumor cells. In agreement with our results, it was mentioned that Oxaphosphinin heterocyclic compounds and their coumarin derivatives possess promising results against leukemia cells [29]. However, our results tested selectivity and cytotoxicity, so tested compounds especially **4a**, **5a**, **11a,b** can be tested for further preclinical and clinical studies. Moreover, compound **5a** (IC_{50}= 39 µM) has a lower IC_{50} than the coumarin-phosphonic analogues that have IC_{50} range from 54-801 µM (Table 1) [29].

Conclusion

Reaction of hexaphenylcarbodiphosphorane **(2)** with α, β-unsaturated compounds 1a,b is used to synthesize different bioactive heterocyclic compounds *via* [4+2] to give the intermediate **3a** then **4a** and **4b** after *Hoffmann degradation*. It can also produce **7a** and **7b** *via* [2+2]-cycloaddition of the carbonyl function in **1a** to the C-P group in **2**. Phosphallene ylide **2** activates the bond of **1b** through [2+2]-cycloaddition to give compound **9**. Among newly synthesized compounds, 4 of them (**4a**, **5a**, **11a, b)** showed promising selective cytotoxicity against human myeloblastic leukemia HL60 cells.

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